THE PROTHALLIA OF ANEIMIA AND LYGODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 136

EDITH MINOT TWISS

(WITH PLATES X AND XI)

A survey of the literature on the prothallia of the Filicineae brings out plainly two points: (1) for a number of years those who have studied this side of the life history of ferns have urged that one must not consider the heart-shaped prothallium, whose development was first worked out, the norm from which all variations are comparatively rare exceptions; (2) many investigators have expressed the conviction that gametophytic characters could be used in classification.

BAUKE (**1**) was among the first to urge this latter idea when, in an effort to determine the position of the Cyatheaceae, he searched the gametophyte for decisive characters to add to those afforded by the sporophyte. The examination of the prothallium of this family convinced him that aid could be gained there, and he extended his observations to the Schizaeaceae and meant to take up other families. Before this work was completed, however, he died.

About ten years after this, GOEBEL (**9**) followed a study of the prothallia of a number of epiphytic ferns with a discussion of the light thrown by them on the possible origin of ferns from algae through bryophytes. In his later work (**11**) he adds to his account of different gametophytes and reviews his former conclusions, ending with the statement that "within the single natural groups also one may well recognize a conformity in the formation of the prothallus which is expressed in the possibility of arrranging them in series." From his investigations in 1896 HEIM (**12**) came to similar conclusions as to the value of gametophytic characters.

It certainly seems worth while to review the situation and see whether something may not be added from this side to the points already made by the study of the sporangial and vascular development of the sporophyte. Any such attempt, however, must be preceded by considerable work in two directions: (1) although the

prothallia of most of the genera have been investigated, a review of the literature shows many points untouched, and much, done some years ago without the aid of modern technique, that needs reinvestigation; (2) much experimental work is yet to be done regarding the effect of different conditions upon the morphology of the thallus. As a beginning I have attempted the study of some of the Schizaeaceae.

Historical résumé

The first report on the prothallium of this group was given by KNY (**13**) at a meeting of the Gesellschaft naturforschender Freunde at Berlin in 1868, where he instanced *Aneimia hirta* as forming a cell plate immediately on emergence of the filament from the spore. BURCK (**6**) confirmed this and added that the apical cell when formed was not, as in Polypodiaceae and Cyatheaceae, at the end of the thallus but at one side.

In 1875 BURCK (**7**) made a more extended study of three species of Aneimia (*A. Phyllitidis*, *A. fraxinfolia*, and *A. longifolia*), and laid considerable stress on the fact that the whole filament, by longitudinal wall formation, takes part in the formation of the thallus, while in polypods this is formed only at the end of the filament. He also finds the formation of the further stages of the thallus from a lateral cell and a later initial group and attempts to trace the course of development here more carefully. As an additional character of considerable importance, he gives what he calls the "pousse laterale normale," a lateral wing formed by some prothallia, which often becomes thickly covered with antheridia.

BAUKE in his paper (**2**) points out that this "pousse laterale normale" is only a lobe of the often very irregular male prothallium. The first point made by BURCK he modifies by saying that in the Polypodiaceae and Cyatheaceae longitudinal division does take place in several cells of the filament, but always after the wall has appeared in the terminal cell, while in Aneimia the cells near the spore divide at the same time or even earlier than those at the distal end. The lateral position of apical cell and initials is still insisted upon, though details as given by BURCK are criticized. Neither investigator finds anything to mention as especially characteristic in the development of the archegonia, and both confirm the account of antheridial devel-

opment given by KNY. He (**14**) reported that the first wall in the antheridium is always flat instead of funnel-shaped, as in the polypods, and that the discharge of sperms takes place through a star-shaped break in the cover cell.

When BAUKE (**3, 4**) continued his investigation of different prothallia, he added *Lygodium japonicum* to those of the Schizaeaceae already examined. Here he pointed out the long-continued persistence of the apical cell. He also found on well-developed prothallia archegonia alone at first and antheridia only when the prothallia were considerably older. The exit of sperms here, he reported, is provided for by the throwing off of the cover cell.

HEIM (**12**), working with the same species of Lygodium, confirmed both of these points. He studied also *Aneimia Dregeana, A. Phyllitidis, A. fraxinfolia,* and *Mohria Caffrorum,* and gives as an additional distinguishing mark of the whole genus warty (*knotige*) thickenings of the side walls of the prothallial cells.

Methods

The soil in which the prothallia used in the present investigation were grown was a mixture of loam and sand. Six-inch pots were filled to within two inches of the top with coarse stones loosely mixed with Sphagnum to facilitate watering and to prevent the dirt from sifting through. One-half an inch of well-packed soil covered this, and over the top a thin layer of soil was sifted through a fine sieve. After sterilization this fineness of the upper soil facilitated the removal of prothallia for study. Pots and soil were sterilized in steam for several hours and, after the sowing of the spores, were kept covered with glass and watered from below. The prothallia were grown in a greenhouse where the temperature was always near 100° F. and lighted with diffuse light from above.

The early stages were obtained free from dirt by sowing the spores on porous clay plates and keeping these in Petri dishes with a little distilled water. Spores were also germinated on the surface of distilled water and of 0.6 per cent. Knopf's solution. Before drawings were made the prothallia were always carefully compared with the same stages on dirt, to determine whether the form or course of cell division had not been altered by the change of medium.

Fungi were kept down by the potassium permanganate solution advised by LANG (**15**). In a letter from Dr. LANG to Dr. COULTER the directions called for a solution decidedly pink; I used with success 0.015^{gr} potassium permanganate in 3.5 liters of water.

Drawings of the early stages were made from living material. The older prothallia were killed with Flemming's weaker solution (**8**), imbedded in paraffin, and the sections stained with safranin and iron alum-hematoxylin.

Lygodium

The species studied was *Lygodium circinatum* (Burm.) Sw. (*L. dichotomum* Sw.), spores of which were obtained through the kindness of Dr. J. N. ROSE, from the botanical gardens at Washington, D. C.

SPORE COATS

The coats reported for the spores of Filicineae are an outer exceedingly delicate epispore, a heavier exine having usually peculiár markings, and a thinner intine which covers the emerging papilla when germination occurs. Paraffin sections of spores of Lygodium, however, showed the possibility of a different situation, and accordingly a study of the development of the coats was undertaken. For this study I was most fortunate in having access to slides prepared by BINFORD (**5**) in his work on the sporangia of *Lygodium circinatum*, and thanks are due him for the aid afforded by his preparations. The slides were stained with safranin and gentian violet, and this should be kept in mind when reading the account of the coloring of the different coats. The sporangia are produced in acropetal succession, so that it is easy to get a clear picture of the different stages.

When the spore mother cell rounds off, the wall is exceedingly delicate, and at the tetrad stage no remnant of it could be identified with any certainty. As *fig. 1* shows, there is a clear space about the tetrad, so that the tapetal protoplasm with its large nuclei does not touch the spores. The protoplasm at the edge of this clear space was carefully examined for traces of the old mother cell wall, but none could be found. Moreover, the same clear space was seen about the spore mother cells themselves in some of the sporangia. Whether the clear space was due to plasmolysis caused by the fixing agent

could not be determined, since there was no opportunity to observe living material.

The wall first formed about the spore is the exine. Very early, before any thickening of this wall has taken place, the color changes from purple to bright red, and the spores increase in size so that the tapetal protoplasm is now close against them. As the exine thickens, a difference in staining becomes apparent, the inner part being red, the outer yellow, but with no clear line of demarkation between them (*fig. 2, ex, r,* and *y*). No chemical tests could be applied to determine the nature of the substances taking these colors, but from what THOMSON (**16**) says of the colors of megaspore membranes of gymnosperms when stained with safranin, and from what he says of the course of development in these membranes, it seems probable that the red indicated the presence of suberin, and the yellow of pectin.

By this time the intine (*fig. 2, i*) has been laid down just within the exine. In the sporangia showing the stage just preceding, the spores were so collapsed that it was impossible to determine whether or not the intine was formed before the exine had differentiated into red and yellow regions.

At a stage shown in *fig. 2* there are here and there in the cytoplasm groups of reddish granules (*fig. 2, g*). These are first recognized as very small, deeply staining bodies at the intersections of the protoplasmic network. They increase in size and the protoplasm, either by the breaking down or drawing together of some of the connecting strands, assumes the appearance of a network of coarser mesh, in which, both at the intersections and along the strands, are the now redly staining granules. The nuclei (*fig. 2, n*) show the beginning of degeneration in the irregular clumping of the chromatin, and later in the disappearance of the membrane, and it is very likely that the nuclear substance contributes largely to the formation of the granules, which take on a deeper and deeper stain as the nuclei become unrecognizable.

In the older sporangia the granules are larger and larger, of fairly regular shape, as if from the rounding up of viscid matter, but of varying size (*fig. 3*). Some of them are close together, and their position suggests that the larger ones may have come from the running together of two or more of the smaller ones. Those in proximity to the spore

coat adhere to it (*fig. 4*), but for some time not very tightly, so that they are easily pulled away in the cutting. The protoplasm comes to form a delicate continuous sheet between the granules and over their surface (*fig. 5*). The mature wall shows that the protuberances thus formed are still of varying sizes and somewhat irregularly placed, and that they and the exposed position of the exine are covered by the thin layer of cytoplasm (*fig. 6*). In the spores of *Lygodium circinatum* (*figs. 7, 8*), sections in which the coats have been broken apart in the cutting show clearly the delicate intine (*i*), the heavy exine (*ex*) with its two differently staining portions, and the epispore (*e*) of heavy projections formed by the tapetal protoplasm. It is probable that in this epispore we have merely a difference in degree from the more delicate one reported for Filicineae. If the granules were not developed, we should have in the thin sheet of protoplasm covering the spores just such an epispore.

DEVELOPMENT OF THALLUS

The exine of the spores is opaque, so that it was difficult to determine just when chlorophyll was formed. The first definite signs of germination are visible in five to seven days, when the spore coats split and a colorless cell, the first rhizoid, protudes (*fig. 9, a*). The first prothallial cell may be seen as a projecting papilla soon after the rhizoid, but seems to grow more slowly, so that by the time it is well out of the spore the rhizoid is five or six times the longer (*fig. 12*). Very shortly there is seen a smaller papilla pushing from the spore at the side of the first (*fig. 10, p^1*), and as the two protrude farther it becomes clear that the smaller one is a cell cut off from the side of the larger, and that the rhizoid has its origin in turn from this smaller cell (*fig. 12*). The first division of the spore does not separate the rhizoid and first prothallial cell.

The chlorophyll grains are fewer in number and smaller than those in the larger cell, where they are crowded in a dense mass about the nucleus. Sometimes two rhizoids appear, as BURCK reports is always the case in *L. japonicum*, but this seems to happen rarely. Even more seldom the prothallial cell emerges before the rhizoid. The three layers of the spore coat may be distinguished at this stage (*figs. 9, 10*). It is not at all unusual for a spore to produce two fila-

ments (*fig. 11*), and the prothallium then is very like a young prothallium of Hymenophyllum or Trichomanes.

Even before the relation of these three cells is clear, division of the larger prothallial cell by a transverse wall has usually taken place (*fig. 10*). Development now proceeds in one of three ways: (*a*) longitudinal and transverse wall formation may follow so as to produce two rows of nearly equal cells (*figs. 13, 14, 15*); (*b*) an oblique wall may take the place of the first longitudinal wall so that, almost from the first, growth is by an apical cell cutting off segments right and left (*figs. 16, 17*); (*c*) the longitudinal walls may not come in at all, and a filament of a single row of cells is produced (*fig. 18*). The last case seems to be comparatively rare; the others appear about equally often.

This course of development may be altered by varying the conditions in which the prothallia grow. Under a screen of potassium bichromate solution, and in both weak and strong sunlight, filaments of a single row of cells were produced, and these did not broaden to a thallus. The filaments sometimes reached a length of 4 or 5^{mm}, attaining this not through numerous divisions, but by the very unusual length of many of the cells. The small cell between the rhizoid and the first prothallial cell was present in these filaments.

In none of these cultures did antheridia appear, though some of them were kept for a time longer than that within which antheridia are produced under usual conditions. The early appearance of antheridia on filamentous prothallia has been reported as usual. WORONEW (**17**), however, says that he failed to get antheridia on filaments growing in weaker light, but was able to bring about their early production by unfavorable conditions of crowding, drying, etc. In a culture growing in dirt badly overrun by algae, I did find a few prothallia with antheridia produced from the third or fourth cell.

Under a screen of ammoniated copper sulfate, the prothallia, while germinating much later and growing more slowly, broadened in the usual way.

In the ordinary cultures, by the end of ten or twelve days an apical cell is established and a thallus is produced, first spatulate and then, by the more rapid growth of the cells on either side of the apical cell,

heart-shaped (*figs. 19–22*). GOEBEL (**11**, p. 204) has called attention to the fact that for a time the wings of such a prothallium are not of the same size. This unequal lobing is commonly the case with *Lygodium circinatum*, as may be seen from *fig. 22*. The apical cell, however, has clearly a terminal position (*figs. 20–22*), and the inequality of lobing seems in this case to come from the faster growth on one side than on the other. Occasionally the two wings seem to develop equally, and in the end they are of equal size in both cases. As BAUKE reports, the apical cell persists for a relatively long time, but finally gives place to several initials.

SEX ORGANS

Antheridia appear in about three weeks, before the apical cell of the prothallium has been succeeded by the group of initials. Any cell of the prothallium may grow out into a papilla which is cut off to form an antheridium. These usually appear on the lower surface, but are found now and then on the upper surface as well. The first wall of the antheridium is often flat (*fig. 23*), as KNY (**14**) reports, but may be so concave as to touch the basal wall (*fig. 24*). There seems to be nothing here, at least in the ordinary forms, that could be called peculiar to the gametophyte of this family. Occasionally, however, more of a stalk is formed in a manner resembling the antheridia of the Osmundaceae (*fig. 25*). One such case is reported by HEIM (**12**).

The formation of a dome-shaped wall and the cutting off of a cover cell follow, and the central cell divides to form a large number of spermatogenous cells; in Lygodium 128 sperms seem to be the characteristic number.

Within six weeks archegonia have appeared. Examination of a number of prothallia at this date showed some with antheridia only, two with archegonia only, and some with both antheridia and archegonia. (All these prothallia were heart-shaped; crowded prothallia of irregular shape are not included.) Of the first, with antheridia only, there were two forms: (*a*) broadly heart-shaped, with antheridia in great numbers over much of the lower surface and crowded near the initials; and (*b*) younger prothallia, with fewer, more scattered antheridia, and none very near the notch. Of the second kind, I

found but two instances. All prothallia examined at a later stage were of the third kind.

The archegonial cushion is somewhat thicker than that usually figured for polypods, and the archegonia project from it in all directions except from the dorsal surface. The development seems to correspond to that reported for polypods (*figs. 26–28*). There are two neck canal nuclei (*fig. 28*); occasionally four are found, but with no walls (*fig. 29*).

Aneimia

SPORE COATS

The sporangia studied for the development of the spore coat were those of *Aneimia hirsuta* (L.) Sw., in which the spores show parallel ridges set with spines. The material, which had been killed in 70 per cent. alcohol and formalin, was imbedded in paraffin and the sections stained, like those of Lygodium, with safranin and gentian violet.

The earlier stages in the formation of the spore coats agree with those in Lygodium except that the mother cell wall is not so delicate, and traces of it may still be seen after the walls of the spores appear in the tetrad. When the exine begins to show differentiation into red and yellow parts, ridges appear on the outer surface and the red is largely confined to these ridges, only a line of it appearing near the inner edge of the coat (*fig. 30*). Stages were found before the one represented and with barely perceptible ridges, and later ones in which they were more prominent. No trace of granules in the cytoplasm can be seen, though the nuclei have an appearance of degeneration, comparable to that in the same stage of Lygodium. A surface view of the coat at this time shows ridges but none of the spines of the mature coat.

That these spines are built up on the ridges by the activity of the tapetal protoplasm seems evident from stages like that shown in *fig. 31*. The exine shows the red and yellow, but the spines stain purple, and they are very easily pulled away from the ridges. Later these spines change their nature, beginning at the part nearest the ridge, and show the yellow stain except just at the tip.

In the case of *Aneimia hirsuta*, then, the epispore consists of these

spines and a general delicate coat of protoplasm. The exine is a coat of two differently staining portions and with ridges on its outer surface. The intine is first clearly seen at the same stage as in Lygodium, when the exine differentiates into red and yellow portions.

FORMATION OF THE THALLUS

The main work was done with *Aneimia Phyllitidis* (L.) Sw., which Dr. BARNES kindly sent from Mexico at the time of his botanical expedition there in 1908. Younger stages were obtained from spores of *A. hirsuta* from material sent to Dr. CHAMBERLAIN from the Philippine Islands, and from those of *A. Phyllitidis*, which Dr. TRELEASE was kind enough to send from the Missouri Botanical Gardens. Spores were also received from Dr. BRITTON from the New York Botanical Garden, but cultures from these were not successful. For the identification of the specimens I am indebted to Dr. JESSE M. GREEMAN of the Field Museum of Natural History, Chicago.

Germination is somewhat slower than in Lygodium and does not take place till the seventeenth or nineteenth day. The rhizoid is first to protrude and the first prothallial cell follows, but the small cell between them does not emerge as it does in Lygodium. It is only when the filament and rhizoid have attained considerable length that it can be seen at all (*fig. 32*).

The filament does not broaden so early or in so regular a manner as in Lygodium. A spatulate and often irregular thallus is formed (*figs. 34, 35*), and within about ten days initials appear at the side (*fig. 36*). From the rapid division of these initials the thallus takes on the heart-shaped form, the lobes being unequal in size. In this case the larger lobe is the original thallus and the smaller lobe is the younger, as GOEBEL reports for Pteris (**11**, p. 205). In prothallia hitherto studied, this inequality is reported as persistent, but while this was true of *A. hirsuta*, it was not for *A. Phyllitidis*, the lobes of this species finally becoming of equal size, as they do in Lygodium. There is here further reason for agreeing with GOEBEL (**11**, p. 205) when he says, "I do not believe that one can construct a phyletic relationship between apical and lateral position of meristem; . . . . in different sections of the Filicineae both occur."

SEX ORGANS

Antheridia appear in about forty days and the points made in the case of Lygodium may be repeated here: The first wall is *not* always flat; instances are occasionally found of several cells in the stalk; and the sperm number (156) is large. The archegonia appear about ten days later, and have two neck canal nuclei, as do those in Lygodium.

Summary

SPORE COATS

The spores of Aneimia and of Lygodium have three distinct coats. The exine is formed first, and as it broadens its composition changes from cellulose, so that with safranin it stains red, and then red and yellow. The change may be from cellulose to suberin, which stains red, and then to suberin and pectin, as pectin stains yellow. The exine of *Aneimia hirsuta* has ridges on the outer surface.

The intine is the second coat formed, and is the one which covers the filament when it emerges from the spore. It remains a delicate cellulose wall.

The epispore is the last to form, and in both Aneimia and Lygodium is produced by the activity of the tapetal protoplasm. In Lygodium granules appear in the protoplasm, increase in size, and adhere to the exine. In *Aneimia hirsuta* spines are formed on the ridges of the exine. The protoplasm forms a thin sheet over these projections and over the intervening surface of the exine.

DEVELOPMENT OF THE THALLUS

The first wall of the spore does not separate the rhizoid and first prothallial cell, but the spore contents divide into two cells of unequal size, from the smaller of which the rhizoid is produced.

The apical cell of Lygodium is terminal, appears early, and is remarkably persistent. In Aneimia it appears later and is lateral.

The lobes of the heart-shaped thallus are at first unequal. In *Aneimia hirsuta* this inequality is permanent, but in *Aneimia Phyllitidis* and in Lygodium the lobes become later of the same size.

SEX ORGANS

The general course of development in both antheridia and archegonia does not differ from that in the Polypodiaceae.

The first wall of the antheridium is not, as reported, always flat, but may be so concave as to touch the basal wall.

The number of sperms is large.

The archegonia have two neck canal nuclei.

Conclusions

There is nothing in the formation of the antheridium or in the unequal lobing of the prothallium that can be considered characteristic of the genera.

The fact that the rhizoid is not produced as a result of the first division of the spore has not yet been reported for other Filicales, and may possibly be peculiar to the Schizaeaceae.

The large number of sperms produced, the occasional stalk of the antheridium, and the frequent occurrence of two prothallial filaments from the spore, are characters which would place the Schizaeaceae with the more primitive families of the Filicineae.

I wish to express my thanks to Dr. JOHN M. COULTER, at whose suggestion this work was undertaken, and to Dr. CHARLES J. CHAMBERLAIN for his kind direction and criticism.

THE UNIVERSITY OF CHICAGO

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EXPLANATION OF PLATES X AND XI

Reference letters: *a*, rhizoid; *e*, epispore; *ex*, exine; *g*, granules which form epispore; *i*, intine; *n*, nucleus; *p*, *p'*, prothallial cells; *r*, red portion of exine; *y*, yellow portion of exine.

PLATE X

Lygodium circinatum

FIG. 1.—Tetrad. ×1050.

FIG. 2.—Portion of spore wall and tapetal protoplasm with granules and nucleus. ×1050.

FIG. 3.—Later stage in the formation of granules. ×1050.

FIG. 4.—Portion of exine with granules adhering to form the epispore. ×1050.

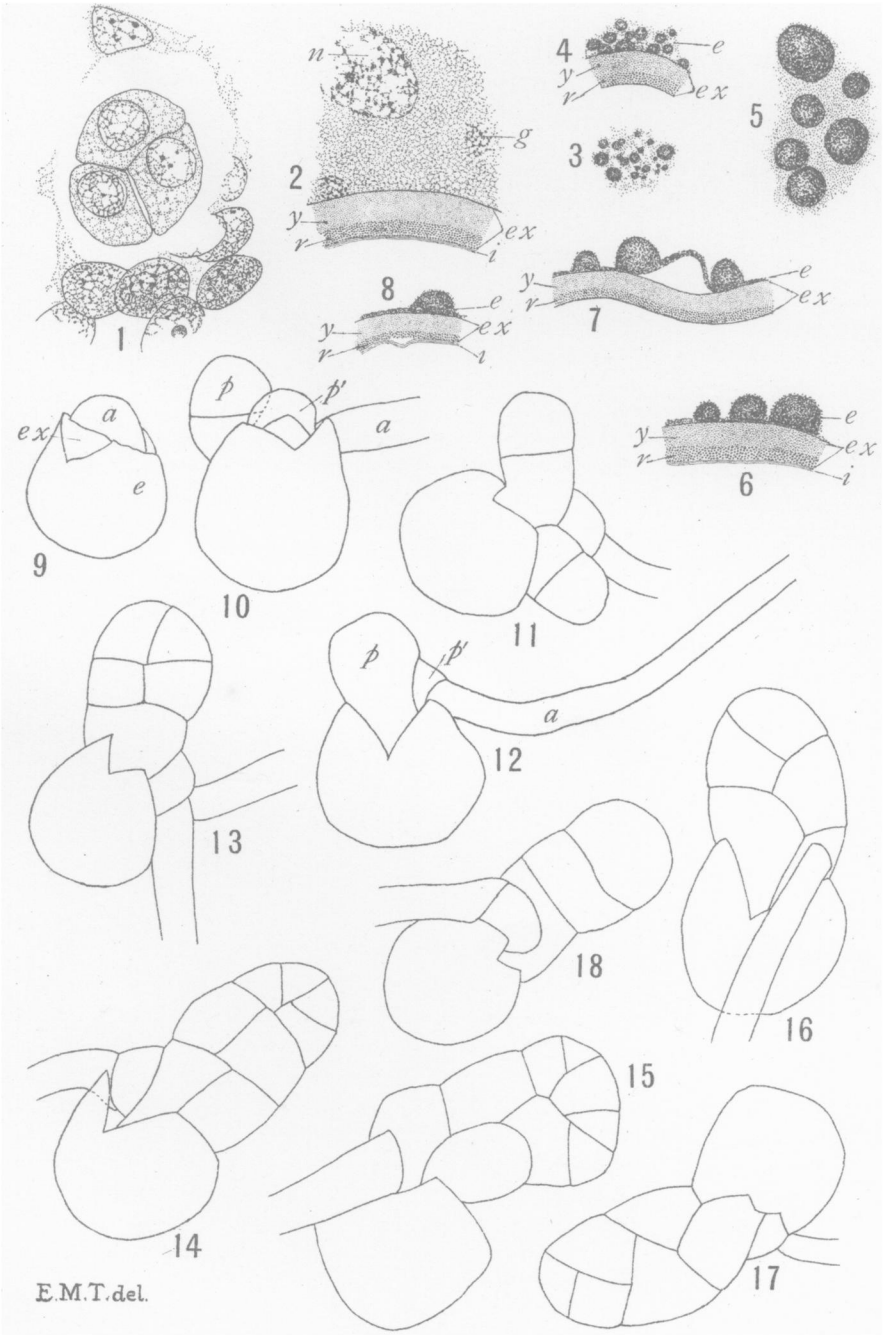
FIG. 5.—Appearance of granules and tapetal protoplasm when spores are mature, showing irregular size and distribution of granules. ×1050.

FIG. 6.—Portion of wall of mature spore, showing epispore with projections of varying size and irregular distribution. ×1050.

FIG. 7.—Portion of spore wall, showing epispore pulled away from the exine; but the view is oblique so that the epispore appears wider than it really is. ×1050.

FIG. 8.—Portion of spore wall with intine pulled away. ×1050.

FIG. 9.—Germination of spore showing rhizoid emerging. ×300.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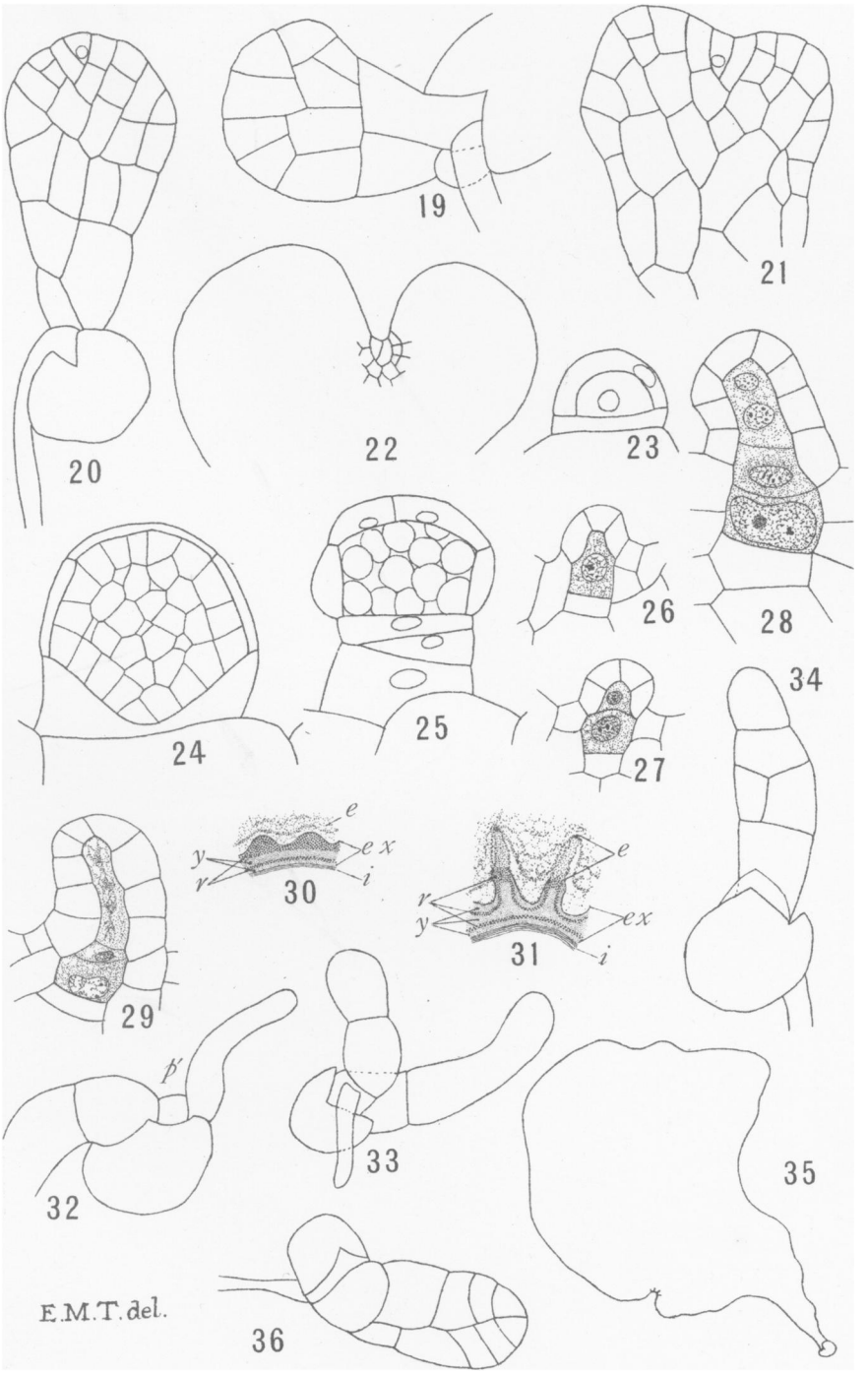
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FIG. 10.—Later stage, showing two papillae (prothallial cells). ×300.

FIG. 11.—Two prothallial filaments coming from one spore. ×300.

FIG. 12.—Further stage, showing relation of prothallial cells and rhizoid. ×300.

FIGS. 13–15.—One method of development of thallus. ×300.

FIGS. 16, 17.—Same stages in thallus developed in another way. ×300.

FIG. 18.—Third way of developing the thallus. ×300.

PLATE XI

Lygodium circinatum

FIGS. 19–22.—Later stages in development of thallus, showing terminal position of apical cell, and in *fig. 22* the unequal size of the lobes. *Fig. 19*, ×300; *figs. 20, 21*, ×200; *fig. 22*, ×50.

FIG. 23.—Young antheridium with first wall flat. ×700.

FIG. 24.—Antheridium at mother cell stage, with first wall concave. ×700.

FIG. 25.—Antheridium with stalk. ×700.

FIGS. 26–28.—Development of archegonium. ×300.

FIG. 29.—Archegonium with remnants of four neck canal nuclei. ×300.

Aneimia hirsuta

FIG. 30.—Portion of spore wall. ×1050.

FIG. 31.—Later stage of same. ×1050.

FIG. 32.—Germination of spore. ×300.

FIG. 33.—Two prothallial filaments from one spore. ×200.

FIG. 34.—Development of thallus. ×300.

FIG. 36.—Still later stage showing lateral position of initials. ×200.

Aneimia Phyllitidis

FIG. 35.—Later stages of development of thallus. ×50.